# USE OF CARNAUBA WAX FOR THE FORMULATION OF ROSMARINIC ACID LOADED SOLID LIPID NANOPARTICLES

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## INTRODUCTION

The inclusion of antioxidants, or rich sources thereof (fruits, aromatic herbs, etc.) in foods is becoming a common procedure of the food industry. Rosmarinic acid (RA) is a phenolic compound with several biological activities, such as antioxidant, anti-inflammatory, antimutagenic, antibacterial and antiviral properties (Parnham and Kesselring, 1985) However, these compounds need protection from the interactions to which they are exposed when incorporated in food matrices. Hence, the formulation of loaded polyphenols nanoparticles can offer a way to protect such compounds from these interactions, in particular with dairy proteins. The use of lipid nanoparticles (SLN) has been extensively reported and represents an alternative carrier system to traditional colloidal carriers (Parhi and Suresh, 2010). Furthermore, SLNs combine advantages such as biocompatibility and biodegradability, physical stability, protection of incorporated compounds, controlled release and specific targeting (Parhi and Suresh, 2010). The increasing demand for functional foods with beneficial effects for human health, has opened the doors to the use of SLN in the food industry, with the incorporation of natural compounds with beneficial purposes.

Thus, SLNs were prepared by a hot melt ultrasonication method using carnauba wax as lipid and Tween 80 as surfactant. The effects of lipid proportion in the lipid mixture and surfactant concentration were evaluated. Also, the stability of the nanoparticles during 28 days was tested in aqueous solution stored at 4 °C, tracking the mean particle size of the different formulations by photon correlation spectroscopy using a ZetaPALS, Zeta Potential Analyzer. Thermal analyses of the nanoparticles were also performed using DSC (Differential Scanning Calorimetry). The loading capacity and loading efficiency were calculated by measuring the concentration of RA in supernatants by HPLC. Surface properties and morphology were observed by Scanning Electron Microscopy (SEM).

## MATERIALS & METHODS

**Preparation of SLNs**
The SLNs were prepared by hot melt ultrasonication method using rosmarinic acid (RA) (0.15 mg/mL) and carnauba wax as lipid and running a $3^2$ factorial design. As surfactant, tween 80 at different percentages (viz. 1, 2 and 3%, v/v) and carnauba wax (0.5, 1 and 1.5%, w/v) were used to optimize the formulation. Carnauba wax was warmed to a temperature 5 °C above the melting point value of the lipid (i.e. 90 °C). The surfactant solution was added to the lipid and polyphenol solution and then homogenized during 1 min at 70% of intensity in sonicator. The resulting solution was left to cool at room temperature. SLNs were stored at 4 °C during 28 days until further use.

**Particle size and zeta potential analyses**
The average particle size (PS), polydispersity (PI) and zeta potential (ZP) were analyzed by phase analysis light scattering using ZetaPALS, Zeta Potential Analyzer (Holtsville, New York, USA); samples were diluted with MilliQ-water to suitable concentration and were carried with an angle of 90 degrees at 25 °C.

**Thermal properties determination**
DSC thermograms of the materials used and final SLNs were obtained using differential scanning calorimetry (DSC-60, Shimadzu, Columbia, USA). The measurements were taken on freeze-dried SLN, and 3 mg of SLN were placed on an aluminum pan and the thermal behavior determined in the range of 20-100 °C at a heating rate of 10 °C/min. Enthalpies were calculated by equipment software.

**Loading efficiency**
The loading efficiency was calculated by measuring the concentration of rosmarinic acid in supernatants by HPLC according the method described by Fonte, *et al.*, (2011). The calculation was performed according to the following formula:

$$LE\% = \frac{\textit{Total amount of Rosmarinic Acid} - \textit{Amount of Rosmarinic Acid on Supernatant}}{\textit{Total amount of Rosmarinic Acid}}$$

**Morphology properties of SLNs**
The morphology of nanoparticles was investigated by SEM (Scanning Electron Microscopy). Briefly, an amount of freeze-dried SLNs were placed in proper supports and coated with gold using a Sputter Coater (Polaron).

## RESULTS & DISCUSSION

**Table 1:** Physical properties of the SLNs throughout storage time, formulated with the different % of lipid (w/v) and percentage of tween 80 (v/v), **(A)** 0.5% : 1%, **(B)** 0.5% : 2%, **(C)** 0.5% : 3%, **(D)** 1.0% : 1%, **(E)** 1.0% : 2%, **(F)** 1.0% : 3%, **(G)** 1.5% : 1%, **(H)** 1.5% : 2%, **(I)** 1.5% : 3%.

| SLN | | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|---|
| Particle size (nm) | 0 | 43 ± 3 | 722 ± 41 | 887 ± 426 | 907 ± 438 | 582 ± 392 | 587 ± 200 | 945 ± 211 | 897 ± 189 | 35 ± 2 |
| | 28 | 124 ± 72 | 892 ± 60 | 542 ± 380 | 585 ± 307 | 438 ± 338 | 527 ± 285 | 605 ± 248 | 491 ± 210 | 49 ± 6 |
| Polydispersity index | | 0,243 | 0,172 | 0,169 | 0,227 | 0,186 | 0,114 | 0,393 | 0,399 | 0,251 |
| Zeta potential | | -6,14 | -5,67 | -1,49 | -1,77 | 0,35 | -6,45 | -4,89 | -9,34 | -7,61 |
| Loading efficiency (%) | | 99,91 | 99,97 | 99,96 | 99,97 | 99,92 | 99,94 | 99,88 | 99,91 | 99,89 |
| Thermal properties | Enthalpy (J/g) | -27,56 | -3,69 | -10,57 | -36,50 | -29,11 | -1,35 | -15,56 | -48,67 | -3,77 |
| | Melting T (°C) | 80,86 | 79,59 | 80,92 | 80,07 | 81,18 | 80,30 | 80,76 | 80,24 | 80,26 |

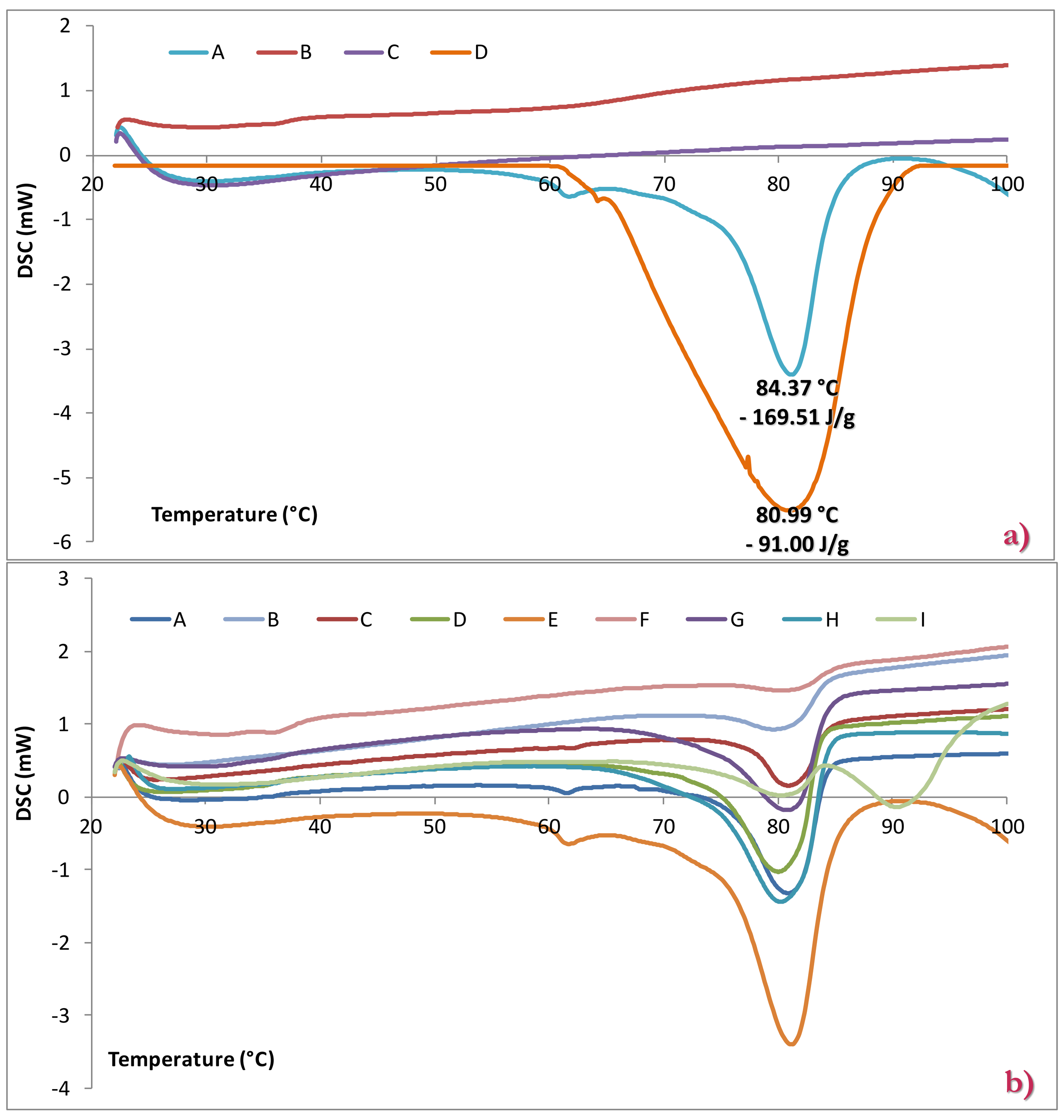


**Fig. 1:** Differential scanning calorimetry thermograms of the ingredients **(a)** and the SLNs **(b)**.
**(a)** Raw materials: **(A)** Carnauba wax **(B)** Rosmarinic acid **(C)** Tween 80 **(D)** 1.0% carnauba wax : 2% Tween 80 without RA.
**(b)** Percentage of carnauba wax (w/v) and percentage of Tween 80 (v/v), **(A)** 0.5% : 1%, **(B)** 0.5% : 2%, **(C)** 0.5% : 3%, **(D)** 1.0% : 1%, **(E)** 1.0% : 2%, **(F)** 1.0% : 3%, **(G)** 1.5% : 1%, **(H)** 1,5% : 2%, **(I)** 1.5% : 3%.

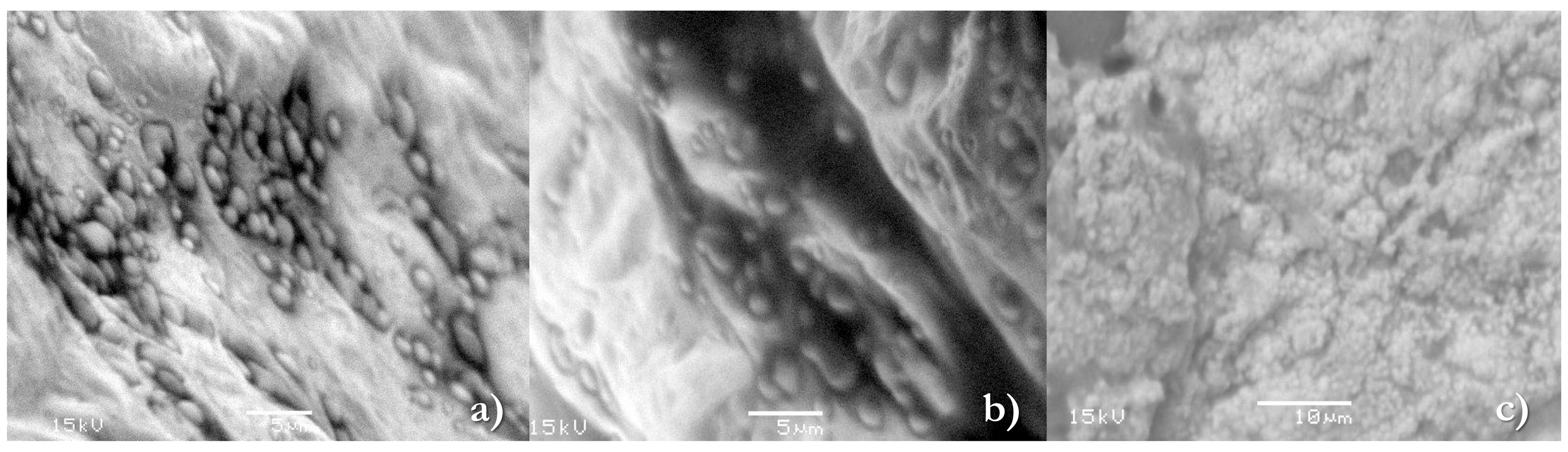


**Fig. 2:** Photographs of SLNs by scanning electron microscope (SEM). Percentage of carnauba wax (w/v) and percentage of Tween 80 (v/v), **(a)** 0.5% : 2%, 5000x **(b)** 1.0% : 2%, 5000x **(c)** 1.5% : 2%, 2000x.

- ✓ In general, increasing lipid content increases the mean particle size, as expected, with exception of formulation I. When using 0.5% of carnauba wax (A, B and C formulations), the average PS increased, with the use of higher contents of tween 80; in contrast, for the other formulations (D-I) with higher lipid %, the average PS decreased when using higher tween 80 content, as expected;
- ✓ There were no significant differences in the PI (P>0.05). Nevertheless, low PI indicates better stability of SLN over time, as examples are formulations B, E and F; in formulations E and I, the PS of SLNs was maintained after 28 days of storage, and with low PI;
- ✓ In general, all formulations resulted in negative ZP, with exception of formulation E, but, all with low absolute values with indicates a possibility of occurrence of aggregation (Muller, 1996). The higher value of ZP was obtained for formulation H, using 1.5% of lipid and 2 % of tween 80, suggesting lower probability of aggregation. In general, these results indicate that a study of a new surfactant has to be considered in the future;
- ✓ The percentage of loading efficiency is high for all formulations (≈ 99.9%), which means that the polyphenol entrapment does not change with the different formulations tested;
- ✓ The melting temperature is similar for all the formulations, range of temperature between 79.6 - 81.2 °C, including the formulation without AR (81.0 ºC; Fig 1aD), but their values were smaller than that for carnauba wax alone (84.4 ºC; Fig 1aB). Formulation I showed a lower enthalpy value, which suggests that the SLNs have lower particle arrangement, in contrast with H, which showed a high value. Even not showing a maintenance of the size after 28 d this formulation is the most stable.
- ✓ Figure 2 shows the SLNs micrographs of chosen formulations. Here it is possible to confirm the cylindrical shape which is characteristic of carnauba wax nanoparticles.

## CONCLUSION

**• The smallest, physical and thermally stable SLNs were those for H formulation (1.5% lipid and 2 % tween 80). The zeta potential was low in absolute values, meaning that possible aggregation could occur in the nanosuspensions. The formulations do not affect the polyphenol entrapment.**

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